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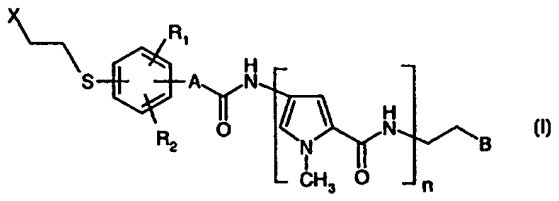
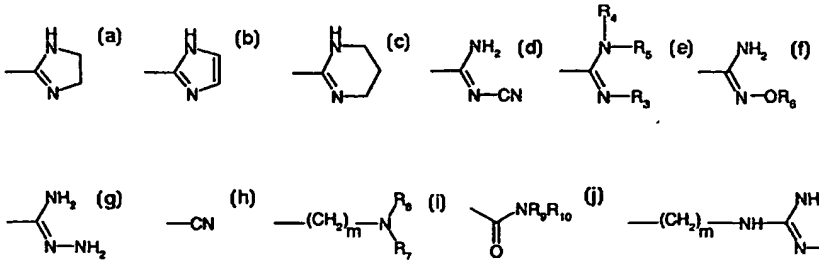


(I)



(57) Abstract

Compounds which are sulfurated distamycin derivatives of formula (I) wherein n is 2, 3 or 4; A is a bond, a C_1–C_4 alkylene or C_2–C_4 alkenylene group; R_1 and R_2, which are the same or different, are selected from hydrogen, C_1–C_4 alkyl optionally substituted by one or more fluorine atoms, and C_1–C_4 alkoxy; X is a halogen atom; B is selected from formulas (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) and (k); wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10}, which are the same or different, are selected from hydrogen or C_1–C_4 alkyl; R_{11} is hydrogen, C_1–C_4 alkyl or hydroxy, and m is 0, 1 or 2; or pharmaceutically acceptable salts thereof; are useful as antitumor agents.

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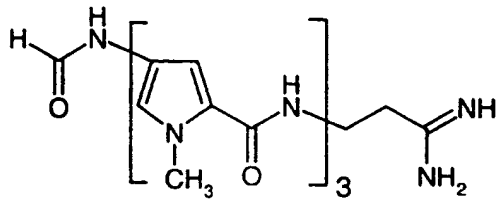
SULFURATED DISTAMYCIN DERIVATIVES, PROCESS FOR PREPARING THEM, AND THEIR USE AS ANTITUMOR AGENTS

The present invention relates to new alkylating antitumor agents analogous to Distamycin A, to a process for their preparation, to pharmaceutical compositions containing them and to their use as therapeutic agents.

Distamycin A, whose formula is reported below



belongs to the family of the pyrroleamidine antibiotics and it is reported to interact reversibly and selectively with DNA-AT sequences, thus interfering with both replication and transcription. See, for a reference, Nature, 203, 1064 (1964); FEBS Letters, 7 (1970) 90; Prog. Nucleic Acids Res. Mol. Biol., 15, 285 (1975).

Several analogous to distamycin are known in the art.

DE-A-1795539 discloses distamycin derivatives in which the formyl group is replaced by a hydrogen atom or by the carboxylic acid residue of a C_1-C_4 aliphatic or cyclopentylpropionic acid.

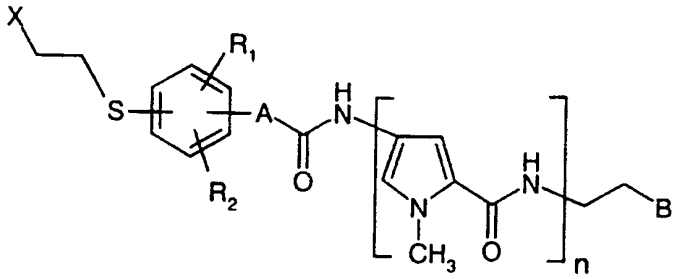
EP-A-246,868 describes distamycin analogues in which the distamycin formyl group is substituted by aromatic, alicyclic or heterocyclic moieties bearing alkylating groups.

WO 97/28123 and WO 97/43258 describe distamycin analogues in which the amidino group is replaced with different nitrogen-containing ending groups and the distamycin formyl group is substituted by an aromatic or a cinnamoyl moiety, respectively.

It has now been found that a new class of distamycin derivatives as defined hereinunder, wherein the distamycin formyl group is substituted by a phenylcarbonyl, phenylalkylcarbonyl or phenylalkenylcarbonyl group bearing a haloethyl-thio group as an alkylating moiety, and the

amidino group is optionally replaced by various nitrogen-containing ending groups, shows valuable biological properties.

Therefore, the present invention provides compounds which are sulfurated distamycin derivatives of formula:



(I)

wherein:

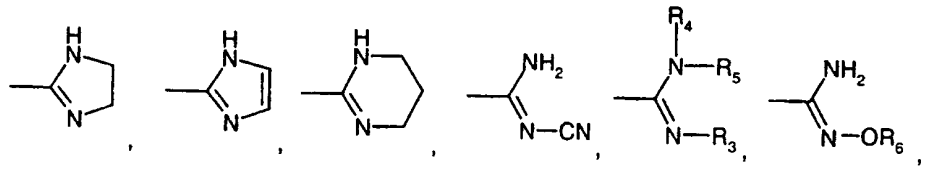
n is 2, 3 or 4;

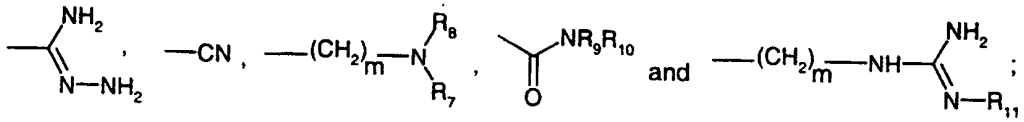
A is a bond, a C_1-C_4 alkylene or C_2-C_4 alkenylene group;

R_1 and R_2, which are the same or different, are selected from hydrogen, C_1-C_4 alkyl optionally substituted by one or more fluorine atoms, and C_1-C_4 alkoxy;

X is a halogen atom;

B is selected from:





wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10}, which are the same or different, are selected from hydrogen or C_1-C_4 alkyl; R_{11} is hydrogen, C_1-C_4 alkyl or hydroxy, and m is 0, 1 or 2;

or pharmaceutically acceptable salts thereof.

The present invention includes within its scope also all the possible isomers covered by the compounds of formula (I), both separately and in admixture, as well as the metabolites and the pharmaceutically acceptable bio-precursors (otherwise known as pro-drugs) of the compounds of formula (I).

In the present description, unless otherwise specified, both terms alkyl and alkoxy include straight or branched C_1-C_4 alkyl and alkoxy groups such as, for instance, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy and tert-butoxy.

Preferred C_1-C_4 alkyl or alkoxy groups are methyl, ethyl, methoxy and ethoxy groups.

When substituted by one or more fluorine atoms, the C_1-C_4 alkyl groups are preferably C_1-C_4 perfluoroalkyl groups, e.g. trifluoromethyl.

Both terms alkylene and alkenylene refer, respectively, to C_1-C_4 alkylene or C_2-C_4 alkenylene groups, as bivalent radicals of the corresponding C_1-C_4 saturated or C_2-C_4 unsaturated hydrocarbons.

Preferred alkylene or alkenylene groups according to the present invention are methylene, ethylene or vinylene groups.

The term halogen atom includes fluorine, chlorine, bromine and iodine, being chlorine and bromine preferred.

Within the compounds of formula (I) the haloethyl-thio group and the A group are in ortho, meta or para position with respect to each other; preferably, the haloethyl-thio and A groups are in meta or para position.

Pharmaceutically acceptable salts of the compounds of formula (I) are their salts with pharmaceutically acceptable either inorganic or organic acids such as, for instance, hydrochloric, hydrobromic, sulfuric, nitric, acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic and p-toluenesulfonic acid.

A preferred class of compounds of the present invention is that wherein, in formula (I):

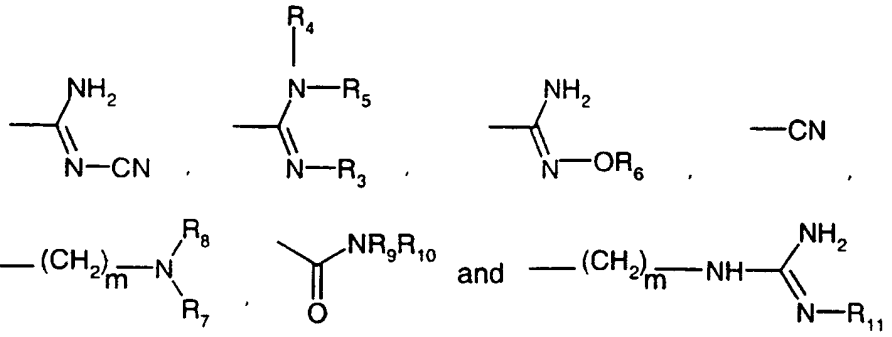
n is 3;

A is a bond or vinylene;

R_1 and R_2 which are the same or different, are selected from hydrogen, methyl, methoxy or trifluoromethyl;

X is chloro or bromo;

B is selected from:



wherein R_3, R_4, R_5, R_7, R_8, R_9, R_{10} and R_{11}, which are the same or different, are selected from hydrogen or methyl; R_6 is hydrogen; and m is 0 or 1;

or the pharmaceutically acceptable salts thereof.

Examples of specific compounds according to the present invention, especially in the form of salts, preferably with hydrochloric acid, are the following:

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N',N'-trimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-

carboxamido]propionamidoxime;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;
2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N-dimethylamine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;
3-[1-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-

carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

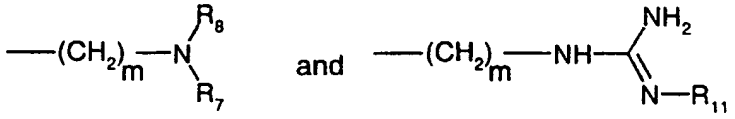
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile; and

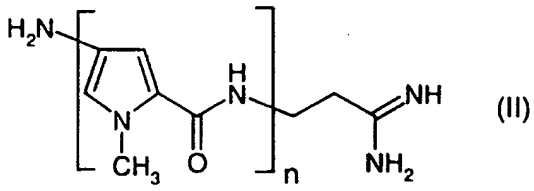
2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine.

A further object of the present invention is a process for preparing the compounds of formula (I), and the pharmaceutically acceptable salts thereof, which process comprises:

(a) when B is other than

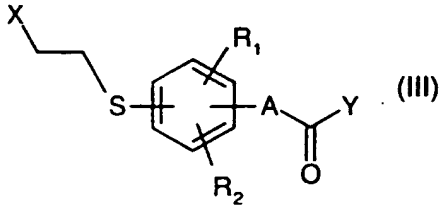


reacting a compound of formula:



(II)

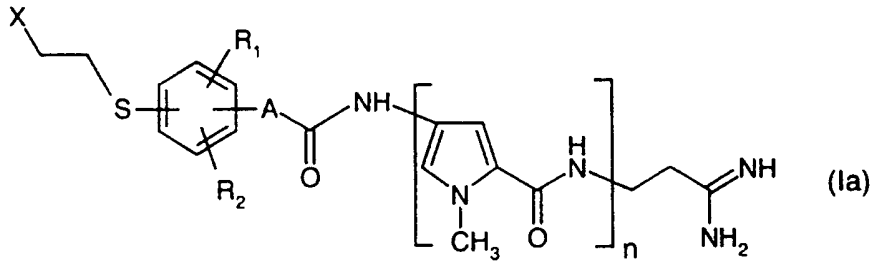
with a compound of formula:



(III)

wherein n, R_1, R_2, X and A are as defined above, and Y is hydroxy or a suitable leaving group;

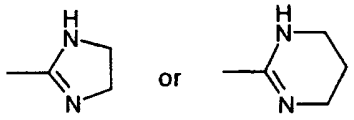
so as to obtain a compound of formula:



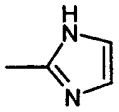
(Ia)

and, then, optionally reacting a compound of formula (Ia) with:

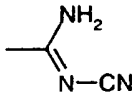
(i) $H_2N-(CH_2)_r-NH_2$, wherein r is 2 or 3, so as to obtain a compound of formula (I) having B equal to:



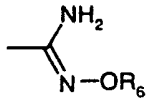
(ii) H_2N-CH_2-CHO, so obtaining a compound of formula (I) having B equal to:



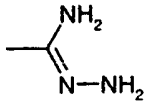
(iii) H_2N-CN, so obtaining a compound of formula (I) having B equal to:



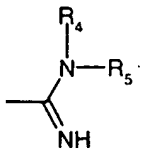
(iv) H_2N-OR_6, so obtaining a compound of formula (I) having B equal to:



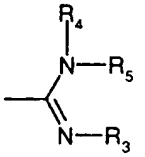
(v) H_2N-NH_2, so obtaining a compound of formula (I) having B equal to:



(vi) HNR_4R_5, so obtaining a compound of formula (I) having B equal to:



and then optionally with H_2NR_3, so obtaining a compound of formula (I) having B equal to:



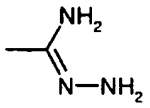
(vii) succinic anhydride, so obtaining a compound of formula (I) having B equal to -C≡N;

(viii) water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-CO-NR_9R_{10}$ wherein R_9 and R_{10} are both hydrogen atoms;

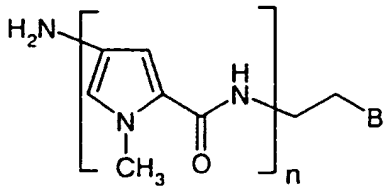
(ix) HNR_9R_{10}, so obtaining a compound of formula (I) having B equal to:

and then with water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-CO-NR_9R_{10}$, wherein R_9 and R_{10} are, each independently, hydrogen or C_1-C_4 alkyl; or

(b) when B is other than

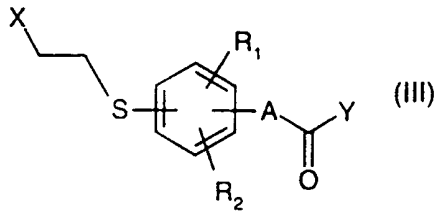


reacting a compound of formula:



(IV)

with a compound of formula:



(III)

wherein n, B, R_1, R_2, X, Y and A are as defined above;
so obtaining the corresponding compound of formula (I);
and, if desired, converting the compound of formula (I) into a pharmaceutically acceptable salt thereof.

In formula (III), Y is hydroxy or a leaving group selected, for instance, from chloro, 2,4,5-trichlorophenoxy, 2,4-dinitro-phenoxy, succinimido-N-oxy, imidazolyl group, and the like.

The condensation reactions as set forth above under processes (a) and (b) is carried out according to known methods, for instance those described in the aforementioned EP-A-246,868.

The reaction between a compound of formula (II) or (IV) with a compound of formula (III) is preferably carried out with a molar ratio (II):(III) or (IV):(III) of from 1:1 to 1:2.

Within the compounds of formula (III) wherein Y is hydroxy, the reaction is carried out in an organic solvent, such as, dimethylsulphoxide, hexamethylphosphotriamide, dimethylacetamide, dimethylformamide, ethanol, phenyl, or pyridine, in the presence of an organic or inorganic base such as triethylamine, diisopropyl ethylamine, or sodium or potassium carbonate or bicarbonate, and of a condensing agent such as, N-ethyl-N'-(3-dimethylamino-propyl)-carbodiimide, N,N'-dicyclohexyl-carbodiimide, or 1-hydroxy-benzotriazole hydrate.

The reaction temperature may vary from about -10°C to about 100°C, and the reaction time from about 1 to about 24 hours.

Within the compounds of formula (III) wherein Y is a leaving group as set forth above, the aforementioned condensation reaction may be carried out in an organic

solvent such as, for instance, dimethylformamide, dioxane, pyridine, tetrahydrofurane, or mixtures thereof with water, optionally in the presence of an organic or inorganic base, e.g. N,N'-diisopropylethylamine, triethylamine, sodium or potassium bicarbonate, at a temperature of from about 0°C to about 100°C, and for a time varying from about 2 hours to about 48 hours.

The reaction between a compound of formula (Ia) according to process (a) and one of the reactants as described above at points (i)-(vi) or (ix), can be carried out according to known methods, for instance those reported in US-4,766,142; WO 97/28123; Chem. Revs. 1961, 155; J. Med. Chem. 1984, 27, 849-857; Chem. Revs. 1970, 151; and "The Chemistry of Amidines and Imidates", edited by S. Patai, John Wiley & Sons, N.Y. (1975).

The reaction of a compound of formula (Ia) with succinic anhydride, as defined in point (vii) above, is preferably carried out with a molar ratio (Ia):succinic anhydride of from 1:1 to 1:3 in an organic solvent such as, for instance, dimethyl sulphoxide or dimethylformamide, and in the presence of an organic or inorganic base such as, e.g., triethylamine, diisopropylethylamine, sodium or potassium carbonate, and the like. The reaction temperature may vary from about 25°C to about 100°C, and the reaction time from about 1 hour to about 12 hours.

The reaction with water in an alkaline medium, as defined in points (viii) and (ix) above, may be carried out according to known methods usually employed for alkaline hydrolysis, for instance by treating the substrate with an excess of sodium or potassium hydroxide in water or in a water/organic solvent admixture, e.g. dioxane, tetrahydrofuran, or acetonitrile, at a temperature of from about 50°C to about 100°C, for a time varying from about 2 hours to about 48 hours.

The compounds of formula (II) are known or may be prepared according to known methods; see, for a reference, Arcamone et al. in Gazzetta Chim. Ital. 97, 1097 (1967).

Also the compounds of formula (III) are known or may be

prepared according to known methods, for instance by working as described in J. Org. Chem. 1993, 58, 4506-4508 or Helvetica Chimica Acta, Vol. 67,(1984), 1316-1327.

The compounds of formula (IV) are known compounds as well, for instance as reported in the aforementioned WO 97/28123.

In view of what above reported, it is clear to the man skilled in the art that when preparing the compounds of formula (I) as set forth above, optional amino groups, i.e. R_7 and/or R_8 of the compounds of formula (IV) equal to hydrogen, need to be properly protected according to conventional techniques, so as to avoid unwanted side reactions.

Likewise, the conversion of the said protected amino groups into the free amines may be carried out according to known procedures. See, for a general reference, J. Org. Chem. 43, 2285, (1978); J. Org. Chem. 44, 811 (1979); J. Am. Chem. Soc. 78, 1359 (1956); Ber. 65, 1192 (1932); and J. Am Chem. Soc. 80, 1154, (1958).

Salification of a compound of formula (I), as well as preparation of a free compound starting from a salt, may be carried out by known standard methods.

Well known procedures such as, e.g., fractional crystallisation or chromatography, may also be followed for separating a mixture of isomers of formula (I) into the single isomers.

The compounds of formula (I) may be purified by conventional techniques such as, e.g., silica gel or alumina column chromatography, and/or by recrystallisation from an organic solvent such as, e.g., a lower aliphatic alcohol, e.g. methyl, ethyl or isopropyl alcohol, or dimethylformamide.

PHARMACOLOGY

The compounds of formula (I) according to the present invention are useful as antineoplastic agents. Particularly, they show cytostatic properties towards tumor cells, so that they can be useful to inhibit growth of various tumors in mammals, including humans, such as, for

instance, carcinomas, e.g. mammary carcinoma, lung carcinoma, bladder carcinoma, colon carcinoma, ovary and endometrial tumors. Other neoplasias in which the compounds of the present invention can find application are, for instance, sarcomas, e.g. soft tissue and bone sarcomas, and the hematological malignancies such as, e.g. leukemias.

The in vitro antitumor activity of the compounds of formula (I) was evaluated by cytotoxicity studies carried out on murine L1210 leukemia cells. Cells were derived from in vivo tumors and established in cell culture. The inhibition of cell growth was determined by counting surviving cells with a Coulter Counter after 48 hours treatment.

The in vitro activity was calculated on concentration-response curves and reported as IC_{50} (concentration inhibiting 50% of the cellular growth in respect to controls) were calculated on dose-response.

The compounds of the invention were tested also in vivo on L1210 murine leukemia and on murine reticulosarcoma M 5076, showing a very good antitumoral activity, with the following procedure.

L1210 murine leukemia was maintained in vivo by i.p. weekly transplantation in CD2F1 female mice, obtained from Charles River Italy. For experiments, 10^5 cells/mouse were injected i.v. in the same strain of mice. Animals were 8 to 10 weeks old at the beginning of the experiments. Compounds were administered i.v. at day +1 after tumor cells injections.

M5076 reticulosarcoma was maintained in vivo by i.m. serial transplantation. For experiments, $5x10^5$ cells/mice were injected i.m. in the same strain of mice. Animals were 8 to 10 weeks old at the beginning of the experiments. Compounds were administered i.v. at day 3, 7 and 11 after tumor injection.

Survival time of mice and tumor growth were calculated and activity was expressed in term of T/C% and T.I.%.

$$T/C = \frac{\text{median survival time treated group}}{\text{median survival time untreated group}} \times 100$$

T.I.= % inhibition of tumor growth respect to control

Tox = number of mice which died for toxicity.
Tox determination was made when mice died before the control and/or tested significant body weight loss and/or spleen and/or liver size reduction were observed.

The compounds of the invention can be administered to mammals, including humans, through the usual routes, for example, parenterally, e.g. by intravenous injection or infusion, intramuscularly, subcutaneously, topically or orally. The dosage depends on age, weight and conditions of the patient and on the administration route. For example, a suitable dosage for administration to adult humans may range from about 0.1 to about 150-200 mg pro dose 1-4 times a day.

Further object of the present invention are pharmaceutical compositions, which comprise a compound of formula (I) as an active principle, in association with one or more pharmaceutically acceptable carrier and/or diluent.

The pharmaceutical compositions of the present invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form. For instance, solutions for intravenous injection or infusion may contain as a carrier, for example, sterile water or preferably, they may be in the form of sterile aqueous isotonic saline solutions.

Suspensions or solutions for intramuscular injections may contain, together with the active compound a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

In the forms for topical application, e.g. creams, lotions or pastes for use in dermatological treatment, the active ingredient may be mixed with conventional oleaginous or emulsifying excipients.

The solid oral forms, e.g. tablets and capsules, may contain, together with the active compound, diluents, e.g., lactose, dextrose, saccharose, cellulose, corn starch and potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents, e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethyl cellulose, polyvinyl-pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates, sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, for instance, lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulation. Said pharmaceutical preparations may be manufactured by known techniques, for example by means of mixing, granulating, tabletting, sugar-coating or film-coating processes.

Further object of the present invention are the compounds of formula (I) for use in a method for treating the human or animal body by therapy.

Furthermore, the present invention provides a method for treating tumors in a patient in need of it, which comprises administering to said patient a composition of the invention.

A further object of the present invention is a combined method for treating cancer or for ameliorating the conditions of mammals, including humans, suffering from cancer, said method comprising administering a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumor agent, close enough in time and in amounts sufficient to produce a therapeutically useful effect.

The present invention also provides products containing a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumour agent as a combined preparation for simultaneous, separate or sequential use in anti-cancer therapy.

The term "antitumor agent" is meant to comprise both a single antitumor drug and "cocktails" i.e. a mixture of

such drugs, according to the clinical practice. Examples of antitumor agents that can be formulated with a compound of formula (I), or alternatively, can be administered in a combined method of treatment, include doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluoro-uracil, melphalan, cyclophosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin, and mitomycin, or mixtures thereof.

The following examples are given to better illustrate the present invention but do not limit the scope of the invention itself.

Example 1

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine

Step I: **The intermediate 4-(2-hydroxyethyl)thiobenzoic acid**

To a solution of 400 mg of 4-thiobenzoic acid in 2.85 ml of NaOH 2N, 0.160 ml of 2-chloroethanol were added. The solution was refluxed for 1 hour, 2.85 ml of hydrochloric acid 2N were then added dropwise and the precipitated was filtered and dried giving 370 mg of a white solid.

FAB-MS: m/z 220, (60, $[M+H]^+$)

PMR ($CDCl_3$) d:

7.61 (d, J= 15.7 Hz, 1H), 7.33 (m, 2H), 6.55 (m, 2H), 6.21 (d, J= 15.7 Hz, 1H), 4.22 (q, J=7.1 Hz, 2H), 3.9 (b.s., 1H), 3.19 (q, J=7.1 Hz, 2H), 1.25 (t, J=7.1 Hz, 3H), 1.28 (t, J=7.1 Hz, 3H).

By analogous procedures and by using the opportune starting materials the following intermediate compounds can be obtained:

3-methyl-4(2-hydroxyethyl)thiobenzoic acid;

4-(2-hydroxyethyl)thiocinnamic acid

FAB-MS: m/z 224

PMR (DMSO-d_6) d:

7.59 (m, 2H), 7.52 (d, J = 16.0 Hz; 1H), 7.31 (m, 2H), 6.46 (d, J= 16.0 Hz, 1H), 4.9 (bs, 1H), 3.57 (t, J=6.8 Hz, 2H),

3.08 (t, J=6.8 Hz, 2H).

Step II: **The title compound**

A solution of 240 mg of the intermediate, as prepared in step I, and 0.7 ml of thionyl chloride in 10 ml of toluene were refluxed for four hours, then the solvent was evaporated in vacuo. The crude residue was dissolved in 20 ml toluene and added portionwise to a solution of 500 mg of 3-[1-methyl-4-[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine dihydrochloride (prepared as reported in J. Med. Chem 32, 774-778, 1989) and 160 mg of potassium bicarbonate in 10 ml of water.

The mixture was vigorously stirred at room temperature for one hour, the solvent was evaporated in vacuo and the crude residue purified by flash chromatography (methylene chloride/ methanol: 85/15) to yield 350 mg of the title compound as a white solid.

FAB-MS: m/z 652, (100, $[M+H]^+$)

PMR (DMSO-d_6) d:

10.34 (s, 1H), 9.98 (s, 1H), 9.92 (s, 1H), 8.9 (b.s., 2H), 8.6 (b.s., 2H), 8.21 (t, J=5.6 Hz, 1H), 7.91 (m, 2H), 7.47 (m, 2H), 7.32 (d, J=1.7 Hz, 1H), 7.24 (d, J=1.7 Hz, 1H), 7.18 (d, J=1.7 Hz, 1H), 7.10 (d, J=1.7 Hz, 1H), 7.06 (d, J=1.7 Hz, 1H), 6.95 (d, J=1.7 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.78 (t, J=7.3 Hz, 2H), 3.48 (m, 4H), 2.60 (t, J=6.5 Hz, 2H).

By analogous procedures and by using the opportune starting materials the following compounds can be obtained:

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N',N'-trimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-

carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N-dimethylamine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine.

Example 2

9) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile

To a solution of 200 mg of 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride (prepared as reported in example 1) in 10 ml DMF were added 60 mg of potassium carbonate and 35 mg of succinic anhydride. The mixture was heated at 60°C for 2 hours. The solvent evaporated under vacuum and the crude residue purified by flash chromatography (methylene chloride/methanol : 8/2) to yield 120 mg of the title compound as a white powder.

FAB-MS: m/z 635, (100, $[M+H]^+$)

PMR (DMSO-d_6) d:

10.30 (s, 1H), 9.96 (s, 1H), 9.91 (s, 1H), 8.31 (t, J=5.7 Hz, 1H), 7.90 (m, 2H), 7.48 (m, 2H), 7.32 (d, J=1.7 Hz, 1H), 7.24 (d, J=1.7 Hz, 1H), 7.20 (d, J=1.7 Hz, 1H), 7.08 (d, J=1.7 Hz, 1H), 7.05 (d, J=1.7 Hz, 1H), 6.93 (d, J=1.7 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.78 (t, J=7.0 Hz, 2H), 3.44 (m, 4H), 2.72 (t, J=6.5 Hz, 2H).

By analogous procedure and by using the opportune starting materials the following compounds can be obtained:

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

FAB-MS: m/z 680, (100, $[M+H]^+$)

PMR ($DMSO-d_6$) d:

10.32 (s, 1H), 9.96 (s, 1H), 9.91 (s, 1H), 9.0 (b.s., 2H), 8.21 (t, J=5.6 Hz, 1H), 7.91 (m, 2H), 7.47 (m, 2H), 7.31 (d, J=1.7 Hz, 1H), 7.23 (d, J=1.7 Hz, 1H), 7.18 (d, J=1.7 Hz, 1H), 7.10 (d, J=1.7 Hz, 1H), 7.06 (d, J=1.7 Hz, 1H), 6.93 (d, J=1.7 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.44 (m, 4H), 3.00 (s, 3H), 2.77 (s, 3H), 2.71 (t, J=6.8 Hz, 2H).

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-

chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N-dimethylamine;

3-[1-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine.

Example 3

14) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine

Step I: **The intermediate 4-(2-chloroethyl)thiocinnamic acid**

To a solution of 150 mg of 4-(2-hydroxyethyl)thiocinnamic acid (prepared as reported in example 1 step I) in 3 ml of pyridine, 0.105 ml of mesyl chloride were added and the solution was warmed for 2 hours at 80°C. The solution was cooled at room temperature and hydrochloric acid 37% was slowly added until pH=1. The precipitate obtained was filtered and washes with water then dried obtaining 100 mg of orange solid.

FAB-MS: m/z 242

PMR ($DMSO-d_6$) d:

12.3 (bs, 1H); 7.63 (m, 2H); 7.54 (d, J = 15.9 Hz, 1H); 7.34 (m, 2H); 6.48 (d, J = 15.9 Hz, 1H); 3.76 (t, J = 7.1 Hz, 2H); 3.40 (t, J = 7.1 Hz, 2H).

By analogous procedure and by using the opportune starting materials the following products can be obtained:

4-(2-chloroethyl)thiobenzoic acid;

FAB-MS: m/z 216

PMR ($CDCl_3$) d:

8.01 (m, 2H); 7.38 (m, 2H); 3.67 (d, J = 7.0 Hz, 2H); 3.35 (d, J = 7.0 Hz, 2H).

4-(2-bromoethyl)thiobenzoic acid;

3-methyl-4-(2-chloroethyl)thiobenzoic acid.

Step II: **The title compound**

A solution of 95 mg of 4-(2-chloroethyl)thiocinnamic acid (prepared as described in step I), 80 mg of dicyclohexylcarbodiimide and 53 mg of 1-hydroxybenzotriazole hydrate in 5 ml of DMF was stirred at 80°C for four hours, cooled at room temperature and then added with 200 mg 3-[1-methyl-4-[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine dihydrochloride (prepared as

reported in J.Med.Chem 32,774-778,1989) and 58 mg of potassium bicarbonate.

The mixture was stirred at room temperature for 2 hours, the solvent was evaporated in vacuum and the crude residue purified by flash chromatography (methylene chloride/ methanol: 8/2) to yield 130 mg of the title compound as a yellow solid.

FAB-MS: m/z 678, (100, $[M+H]^+$)

PMR (DMSO-d_6) d:

10.23 (s, 1H), 9.96 (s, 1H), 9.91 (s, 1H), 8.9 (b.s., 2H), 8.6 (b.s., 2H), 8.21 (t, J=5.6 Hz, 1H), 7.55 (m, 2H), 7.46 (d, J=75.8 Hz, 1H), 7.41 (m, 2H), 7.30 (d, J=1.7 Hz, 1H), 7.23 (d, J=1.7 Hz, 1H), 7.17 (d, J=1.7 Hz, 1H), 7.01 (d, J=1.7 Hz, 1H), 6.96 (d, J=1.7 Hz, 1H), 6.95 (d, J=1.7 Hz, 1H), 6.77 (d, J=15.8 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.76 (t, J=7.3 Hz, 2H), 3.49 (m, 2H), 3.40 (t, J=7.3 Hz, 2H), 2.60 (t, J=6.4 Hz, 2H).

By analogous procedure and using the opportune starting material the following product can be obtained:

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-

carboxamido]propionitrile;
2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine.

Example 4

Tablets each weighing 0.250 g and containing 50 mg of the active substance can be manufactured as follows:

Composition for 10,000 tablets	
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride	500 g
Lactose	1,400 g
Corn starch	500 g
Talc powder	80 g
Magnesium stearate	20 g

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride, lactose and half of the corn starch were mixed; the mixture was then forced through a sieve of 0.5 mm mesh size.

Corn starch (10 g) was suspended in warm water (90 ml) and the resulting paste was used to granulate the powder. The granulate was dried, comminuted on a sieve of 1.4 mm mesh size, then the remaining quantity of starch, talc and magnesium stearate was added, carefully mixed and processed into tablets.

Example 5

Capsules, each dosed at 0.200 g and containing 20 mg of the active substance can be prepared as follows:

Composition for 500 capsules	
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride	10 g
Lactose	80 g
Corn starch	5 g
Magnesium stearate	5 g

This formulation can be encapsulated in two-piece hard gelatin capsules and dosed at 0.200 g for each capsule.

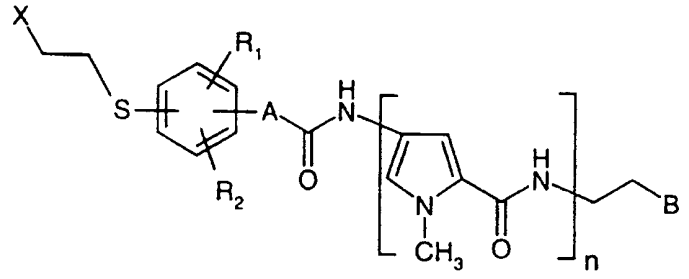
Example 6

Intramuscular Injection 25 mg/ml

An injectable pharmaceutical composition can be manufactured by dissolving 25 g of 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride in sterile propyleneglycol (1000 ml) and sealing ampoules of 1-5 ml.

CLAIMS

1. A compound which is a sulfurated distamycin derivative of formula:



(I)

wherein:

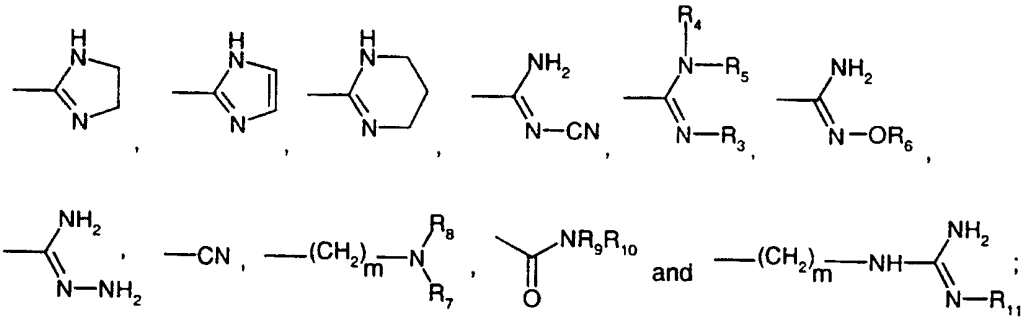
n is 2, 3 or 4;

A is a bond, a C_1-C_4 alkylene or C_2-C_4 alkenylene group;

R_1 and R_2, which are the same or different, are selected from hydrogen, C_1-C_4 alkyl optionally substituted by one or more fluorine atoms, and C_1-C_4 alkoxy;

X is a halogen atom;

B is selected from:



wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10}, which are the same or different, are selected from hydrogen or C_1-C_4 alkyl; R_{11} is hydrogen, C_1-C_4 alkyl or hydroxy, and m is 0, 1 or 2;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10} and R_{11} are, independently from each other, hydrogen, methyl or ethyl.

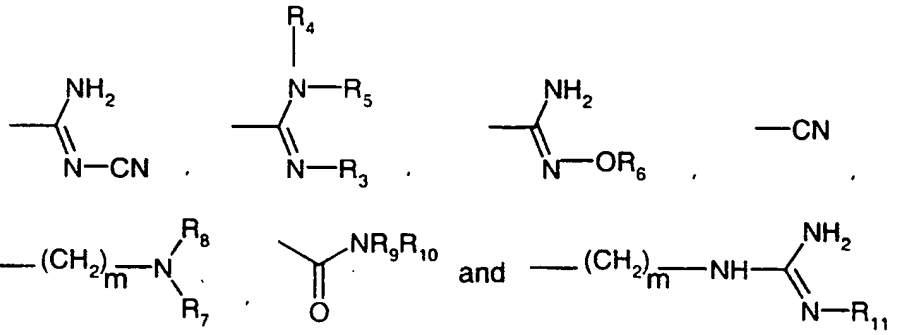
3. A compound according to claim 1 or 2 wherein n is 3;

A is a bond or vinylene;

R_1 and R_2 which are the same or different, are selected from hydrogen, methyl, methoxy or trifluoromethyl;

X is chloro or bromo;

B is selected from:



wherein R_3, R_4, R_5, R_7, R_8, R_9, R_{10} and R_{11}, which are the same or different, are selected from hydrogen or methyl; R_6 is hydrogen; and m is 0 or 1; or a pharmaceutically acceptable salt thereof.

4. A compound selected from the group consisting of:

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N',N'-trimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-

carboxamido]propion-N-cyanamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;
2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N-dimethylamine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-

bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylthio)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

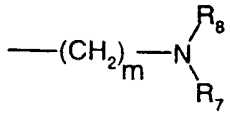
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylthio)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;

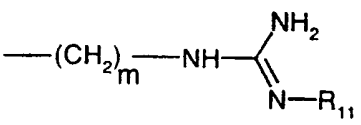
and the pharmaceutically acceptable salts thereof.

5. A process for preparing a compound of formula (I) as defined in claim 1, which process comprises:

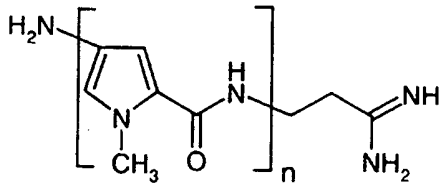
(a) when B is other than



and

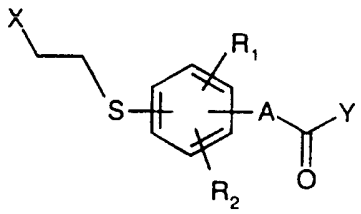


reacting a compound of formula:



(II)

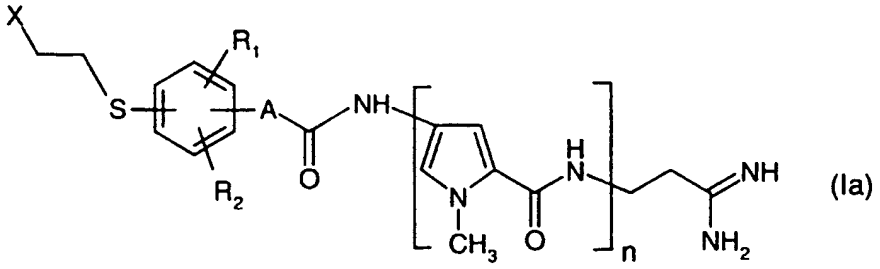
with a compound of formula:



(III)

wherein n, R_1, R_2, X and A are as defined in claim 1, and Y

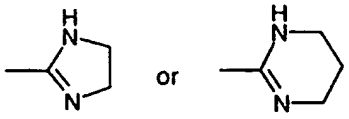
is hydroxy or a suitable leaving group;
so as to obtain a compound of formula:



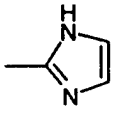
(Ia)

and, then, optionally reacting a compound of formula (Ia) with:

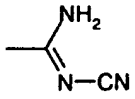
(i) $H_2N-(CH_2)_r-NH_2$, where r is 2 or 3, so as to obtain a compound of formula (I) having B equal to:



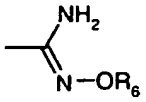
(ii) H_2N-CH_2-CHO, so obtaining a compound of formula (I) having B equal to:



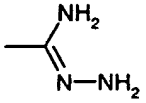
(iii) H_2N-CN, so obtaining a compound of formula (I) having B equal to:



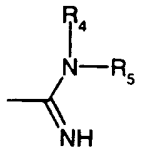
(iv) H_2N-OR_6, so obtaining a compound of formula (I) having B equal to:



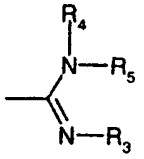
(v) H_2N-NH_2, so obtaining a compound of formula (I) having B equal to:



(vi) HNR_4R_5, so obtaining a compound of formula (I) having B equal to:



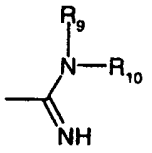
and then optionally with H_2NR_3, so obtaining a compound of formula (I) having B equal to:



(vii) succinic anhydride, so obtaining a compound of formula (I) having B equal to -C≡N;

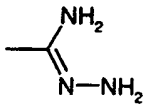
(viii) water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-CO-NR_9R_{10}$ wherein R_9 and R_{10} are both hydrogen atoms;

(ix) HNR_9R_{10}, so obtaining a compound of formula (I) having B equal to:

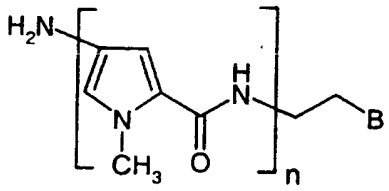


and then with water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-CO-NR_9R_{10}$, wherein R_9 and R_{10} are as defined in claim 1; or:

(b) when B is other than

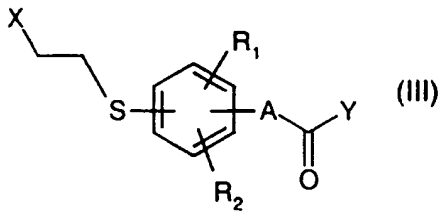


reacting a compound of formula:



(IV)

with a compound of formula:



(III)

wherein n, B, R_1, R_2, X, Y and A are as defined above; so obtaining the corresponding compound of formula (I) and, if desired, converting the compound of formula (I) into a pharmaceutically acceptable salt thereof.

6. A process according to claim 5 wherein, in the compounds of formula (III), Y is hydroxy or a group selected from chloro, 2,4,5-trichlorophenoxy, 2,4-dinitrophenoxy, succinimido-N-oxy and imidazolyl.

7. A pharmaceutical composition comprising one or more pharmaceutically acceptable carriers and/or diluents and, as the active principle, a compound as defined in claim 1.

8. A compound as defined in claim 1 for use in a method of treatment of the human or animal body by therapy.

9. A compound as defined in claim 8 for use as an antitumor agent.

10. Use of a compound as defined in claim 1 in the manufacture of a medicament for use as an antitumor agent.

INTERNATIONAL SEARCH REPORT

International Application No
PC1/EP 99/05348

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D207/34 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 43258 A (PHARMACIA & UPJOHN S.P.A.) 20 November 1997 (1997-11-20) cited in the application claims 1,2,4-10	1-3,5, 7-10
A	WO 97 28123 A (PHARMACIA & UPJOHN S.P.A.) 7 August 1997 (1997-08-07) cited in the application claims 1-3,5-11	1-3,5, 7-10
A	WO 97 03957 A (PHARMACIA & UPJOHN S.P.A.) 6 February 1997 (1997-02-06) claims 1,2,5-9	1-3,5, 7-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 November 1999	12/11/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/05348

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	F. M. ARCAMONE ET AL.: JOURNAL OF MEDICINAL CHEMISTRY, vol. 32, no. 4, 1989, pages 774-8, XP000608784 cited in the application page 775, compound 4; page 776, table II; pageb 777, right-hand column, lines 35-52 ---	1-3,5,8, 9
A	EP 0 246 868 A (FARMITALIA CARLO ERBA S.P.A.) 25 November 1987 (1987-11-25) cited in the application claims 1,2,5-9 ---	1-3,5, 7-10
A	WO 94 20463 A (MENARINI INDUSTRIE FARMACEUTICHE RIUNITE S.R.L.) 15 September 1994 (1994-09-15) claims 1,2,9-12 ---	1,5,7-10
A	DD 297 638 A (FARMITALIA CARLO ERBA S.R.L.) 16 January 1992 (1992-01-16) claims 1,2,7,8 ---	1,7-10
A	US 5 395 849 A (M. D. WITTMAN ET AL.) 7 March 1995 (1995-03-07) claims 1,7-10,12; examples 1-4 -----	1,7-10

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/05348

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9743258 A	20-11-1997	AU 2701697 A	05-12-1997
		EP 0912509 A	06-05-1999
		NO 985307 A	12-01-1999
		PL 329878 A	12-04-1999
WO 9728123 A	07-08-1997	AU 1596097 A	22-08-1997
		CA 2244139 A	07-08-1997
		EP 0880499 A	02-12-1998
WO 9703957 A	06-02-1997	AU 6357996 A	18-02-1997
		BR 9606528 A	23-12-1997
		CA 2199635 A	06-02-1997
		CN 1159183 A	10-09-1997
		EP 0787126 A	06-08-1997
		HU 9702393 A	28-04-1998
		JP 10506410 T	23-06-1998
		NO 971142 A	12-03-1997
		PL 319352 A	04-08-1997
EP 246868 A	25-11-1987	AT 80617 T	15-10-1992
		AU 597659 B	07-06-1990
		AU 7316387 A	26-11-1987
		BG 60531 B	28-07-1995
		CA 1314551 A	16-03-1993
		CS 9104137 A	16-09-1992
		DE 3781716 A	22-10-1992
		DK 254587 A	21-11-1987
		FI 872173 A,B,	21-11-1987
		GR 3006163 T	21-06-1993
		HK 31993 A	08-04-1993
		IE 60198 B	15-06-1994
		IL 82553 A	10-06-1991
		JP 1898111 C	23-01-1995
		JP 6023193 B	30-03-1994
		JP 62294653 A	22-12-1987
		KR 9511408 B	04-10-1995
		MX 9203122 A	01-07-1992
		NZ 220361 A	26-04-1990
		PT 84896 A,B	01-06-1987
		SG 3793 G	12-03-1993
		SU 1528316 A	07-12-1989
		US 5017599 A	21-05-1991
		US 5049579 A	17-09-1991
		US 5310752 A	10-05-1994
		ZA 8703593 A	12-11-1987
WO 9420463 A	15-09-1994	IT 1271456 B	28-05-1997
		AU 6206894 A	26-09-1994
		CA 2157187 A	15-09-1994
		EP 0690840 A	10-01-1996
		JP 8508720 T	17-09-1996
DD 297638 A	16-01-1992	NONE	
US 5395849 A	07-03-1995	NONE	

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